ESTERS PRODUCED FROM n-HEPTADECANE BY MICROCOCCUS CERIFICANS

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Prior reports from these laboratories have described esters produced from alkanes (having even numbers of carbon atoms) and from 1-alkenes by Micrococcus cerificans (Stewart et al., 1959; Stewart and Kallio, 1959; Stewart et al., 1960). Invariably, these esters had an alcohol moiety with a carbon skeleton identical to that of the hydrocarbon serving as growth substrate and the acid moiety was predominantly (but not invariably) palmitic acid regardless of the carbon skeleton of the substrate. In attempting to extend and further analyze these ester patterns the organisms were grown on odd-numbered n-alkanes in chemically defined media and the culture fluids worked up as previously described. Attempts to isolate products from culture fluids of the organisms growing at the expense of n-undecane, n-tridecane and n-pentadecane were uniformly unsuccessful. Material responding positively to the hydroxamic acid test was isolated from cultures of M. cerificans grown on n-heptadecane and mass analysis reveals some additions to the general pattern of ester production by the bacterium found in the case of even carbon number alkanes.

Heptadecane was synthesized from octadecanol-1 by hydrogenolysis with palladium as catalyst. The mass analysis of n-heptadecane so produced was:

Alkane/specimen	% M
nC_{16}	0.8
nC_{17}	>98.
nC_{18}	0.3
nC_{19}	<0.1

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General methods for growth, isolation of product and mass analysis have been described (Stewart et al., 1959).

In the "high" molecular weight range the mass spectrum of the product from n-heptadecane is characterized by three ions of m/q 508, 494, and 480 in the relative intensities, 78:9:12, respectively. The molecular weights of these ions were established by recording the spectrum before and after the addition of an authentic sample of cetyl palmitate (m/q = 480). In view of the fact that in the primary fragment portion of the mass spectrum there are found the ions with m/q = 271 ($C_{16}H_{33}CO_2H_2^+$), 257 ($C_{15}H_{31}CO_2H_2^+$) and 243 ($C_{14}H_{29}CO_2H_2^+$) in the relative intensities 77:10:12, respectively, we interpret the mass spectrum as indicating the product to be a mixture of three esters,

heptadecyl pentadecanoate	-12%
heptadecyl palmitate	-9-10%
heptadecyl margarate	-77-78%

That the esters contain the heptadecyl moiety is shown by the fragment peak of m/q = 238 - $C_{17}H_{34}^+$, and the absence of the hexadecyl (cetyl) group follows from the essential absence from the mass spectrum of m/q = 224 - $C_{16}H_{32}^+$ that is an important fragment peak in the mass spectrum of cetyl palmitate. The intensity of the ion m/q = 224 relative to that of m/q = 257 indicated that less than 1/4 of the material called heptadecyl pentadecanoate can be cetyl palmitate. Although there is a fragment ion at m/q = 210 - $C_{15}H_{30}^+$, this <u>does not</u> indicate the presence of a pentadecyl ester, rather it is afragment to be expected from a heptadecyl ester since a characteristic fragment ion of the cetyl palmitate mass spectrum is the one with m/q = 196 - $C_{14}H_{28}^+$. Additional support for the presence of the three acid moieties, pentadecoic, palmitic and margaric, is found in the presence in the mass spectrum of the ions of m/q = 225 ($C_{14}H_{29}CO^+$), m/q = 239 ($C_{15}H_{31}CO^+$) and m/q = 253 ($C_{16}H_{33}CO^+$) in approximately the same relative intensities as the more characteristic, corresponding protonated acid ions ($RCO_2H_2^+$) at m/q = 243, 257, 271 mentioned above.

It is of interest to note that the mass spectrum of this product from heptadecane has a fragment ion at m/q = 283 ($C_{17}H_{35}COOCH_2^+$) corresponding to the fragment found at m/q = 269 ($C_{16}H_{33}COOCH_2^+$) of the cetyl palmitate mass spectrum.

It should be further noted that whereas the cetyl palmitate mass spectrum has the fragment ion of m/q = 213 ($C_{14}H_{29}O^+$?) the mass spectrum of the ester mixture from the heptadecane has the homologous fragment ion m/q = 227 ($C_{15}H_{31}O^+$), a behavior analogous to that noted above with respect to the fragments m/q = 196 ($C_{14}H_{28}^+$) of cetyl palmitate and 210 ($C_{15}H_{30}^+$) of the heptadecane ester. Both 213 and 196 are absent from the heptadecane-ester mass spectrum.

Clearly, none of the products could arise from the minimal impurities present in the substrate and must therefore represent materials constructed from n-heptadecane. Heptadecyl margarate probably represents a case of C_1 alkane oxidation and subsequent "direct" esterification of a normal fatty acid and alcohol similar to the case of cetyl palmitate production from n-hexadecane (Stewart et al., 1959). Direct evidence for this mechanism of cetyl palmitate formation by M. cerificans has been obtained with hexadecane-1-C^{14} (Finnerty and Kallio, unpublished observation). Formation of heptadecyl pentadecanoate may be formulated along the same lines by including the removal of an acetyl group from the fatty acid prior to esterification (Webley et al., 1956).

The origin of heptadecyl palmitate is more difficult to interpret. Two possibilities appear feasible. It seems possible that a fatty acid peroxidase is responsible for some α-oxidation of the margaric acid produced prior to ester synthesis (Stumpf, 1956; Martin and Stumpf, 1959). Alternatively it might be suggested that palmitate is synthesized de novo from two carbon fragments although analysis of myristyl palmitate formed by the organism from tetradecane-1-C^{14} suggests this is not the case.

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